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Exhaled nitric oxide as a marker in asthma

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This section will describe how nitric oxide (NO) is formed in the body, its presence in exhaled air, and what usefulness the measurement of this might have in the treatment of asthma. The formation of considerable amounts of NO in the nose and paranasal sinuses means that there are demands on the measurement technique for exhaled NO, if its measurement is to provide information on the status of the lower airways. The general biology of exhaled NO has recently been reviewed [1–4], and the reader is referred to these papers for many aspects that cannot be covered in the present synopsis. Medline (July 1997) now contains more than 110 references on NO and exhalation.

Oxides of nitrogen have until recently been regarded as environmental pollutants, consisting of molecules such as NO, NO_2, N_2O_4. Their abundance, chemistry and impact on health including asthma has recently been discussed in a review [5]. One of the above molecules, NO has been found to be formed in the body and to be a principal signalling molecule between vascular endothelium and smooth muscle as well as between cells in the nervous system [6–8]. NO exerts its effects through stimulation of the enzyme guanylate cyclase, the activity of which is increased up to 20-fold when NO is bound to the haeme ring of this enzyme. This is in much the same way as oxygen is bound to haemoglobin [9].

Several other NO-containing molecules have been suggested as alternatives to NO in explaining bioactivity similar to endothelium-derived relaxing factor (EDRF), the original bioactivity identified first as NO. However, both in the central and peripheral nervous systems and in blood vessels, authentic NO rather than *e.g.* a nitrosothiol has a characteristic pattern [10–12]. Evidence for formation of intact NO in mammals has been found through its presence in exhaled air [13–15]. The origin of exhaled NO might be theoretically from either: endothelial formation; from NO synthesis in nerves; or from NO synthase produced by an inducible form of the NO synthase, *e.g.* in macrophages or neutrophils. Formation of NO and the presence of different types of NO synthases have been suggested or demonstrated in a number of cells in contact with the airways, including the respiratory epithelium itself [16] (see also below).

NO synthase in nerves including those of the human has been suggested as a powerful bronchial relaxing mechanism [17]. NO is also formed upon activation of the immune defence, especially by endotoxins, and parts of the symptoms of the involved inflammatory response are likely to involve novel synthesis of NO by inducible nitric oxide synthase [6]. When nitric oxide is formed as a signalling molecule this is achieved by either of two constitutive enzyme forms, endothelial NO synthase (eNOS) or neuronal NO synthase (nNOS). These enzymes form NO in a calcium-dependent fashion, where calcium entry into the NO-forming cell is triggered by stretching of the cell or by the action of a signalling substance on cell surface receptors. Indeed, stretch of the lung and its airways may cause increased exhalation of NO [18, 19].

During the inflammation of specific or nonspecific immune responses a specific form of NO synthase is synthesized *de novo* due to gene activation. This type of inducible enzyme forms NO in a calcium-independent way and is often called iNOS. Three NO synthase enzymes have been isolated, their amino acid sequence and gene localizations determined. They constitute three distinct gene products [20, 21]. All three types of NO synthase have been demonstrated in airways epithelium [22–26]. Very high expression of messenger ribonucleic acid (mRNA) for the inducible form of NO synthase has been found in both lower airways and paranasal sinuses of the human [27, 28]. Thus, it seems very likely that the respiratory epithelium is an important source of exhaled NO.

Exhaled NO is increased in experimental asthma in animals [29] as well as in humans [30–33]. In humans, an increased exhalation of NO is seen in the late phase of provoked asthma [34] and in patients with established asthma and symptoms [30–33, 35]. It has been reported that NO does not increase in the early phase of asthma [34], but in this study a measurement technique was used (slow exhalation over nearly a full vital capacity) which does not exclude nasal gas being added to exhaled air. Thus, smaller changes in the initial phase might have been overlooked, and the question of whether NO is increased early in the asthmatic attack needs further study. In animals NO is increased, followed by a decrease, during the early phase of an acute bronchial obstruction [29]. Endogenous NO seems to exert inhibitory effects on both responses to mediators and to allergens in humans as well as in animals [36–39], but inhibition of NO synthase does not alter baseline forced expiratory volume in one second (FEV_1) in asthmatic subjects [40, 41], and inhalation of methacholine or salbutamol does not seem to alter exhaled NO in humans [35]. In respect of its bronchodilator role, inhaled NO is a bronchodilator in both humans as well as in animals [42–44]. Despite this, it may be that exhaled NO is increased in asthmatics as a result of expression of inducible NO synthase [41] and that this NO formation might have detrimental effects [45]. Regardless of whether

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endogenous NO has a protective or a detrimental role in asthma and other abnormal lung conditions, exhaled NO is a biochemical indicator of the function of the respiratory system which will prove to be useful since it can indicate an ongoing condition even after the acute changes detected by classical lung function tests, have subsided. It is also a noninvasive test which has already proven useful, *e.g.* in measurements in children [46–49]. Exhaled NO will be useful for monitoring not only disease but also to establish the effectiveness of and compliance with asthma treatment, and will possibly lead to better individual adjustment of treatment dosage [50], an especially desirable aspect in children.

When NO is exhaled it occurs as the result of formation from L-arginine. The NO is produced locally in the respiratory system, as it is present in isolated perfused lungs and is not decreased by inhibiting the circulation in anaesthetized animals [13, 51, 52]. Exhaled NO originates, to a considerable extent, in the conducting airways, since it occurs with an early peak appearing before carbon dioxide has reached its maximum [53]. A large contribution to this airway NO can come from the nose and pharynx [28, 30, 54–56]. Nevertheless, in intubated humans a significant NO excretion rate is found [56], and tracheal NO concentrations are elevated in asthma [57]. Contribution from nasal NO can, in awake subjects, be minimized by exhalation against special or commercially available resistors [58, 59]. Exhaled NO concentration is inversely related to ventilation, and its excretion rate increases both during nonexercise hyperventilation and during exercise [53]. Breathing against a resistor yields reproducible exhaled NO concentrations [58, 59]. Breathing against a resistor might thus add the advantage that the exhalation rate is more easily controlled, but may introduce the possibility of effects as a result of stretching of the lung. Instead of flow control by a resistor, variations in exhalation rate may be measured by continuous-flow determination during single-breath exhalation without a resistor, and the excretion of NO calculated, on-line, from the exhalation rate and NO concentration, discarding the first part of possibly nasally contaminated exhaled air. The latter approach may have considerable advantages in patients with asthma or other types of respiratory incapacitation, where prolonged exhalation times are difficult to cope with.

Conclusions

Clearly, NO is an interesting marker in asthma, where high levels are reached especially during the later phases of asthmatic reactions or in more severely afflicted patients. Further research is necessary until the enzymatic origin of exhaled NO can be determined, but at the moment it constitutes an on-line measurement of lung (conducting airways and parenchyma) function. Since smokers exhibit decreased NO excretion [32] it is obvious that the determination of both increments and decrements of exhaled NO will be of interest. There is a need for standardization of methods with, as far as possible, exclusion of nasal gas from the exhaled air. It is desirable to use single breath methods which can distinguish between early exhalate with a possibility of nasal gas contamination, and later portions which better represent samples of the lower airways. The later phase of exhalation exhibits a plateau which can be estimated even during single-breath conditions, and which likely represents NO added from the lower airways during exhalation [3, 53, 58, 59]. NO formation by the lungs is stimulated by stretch [18, 19]. Exhaled NO is also affected, but in a negative fashion, by carbon dioxide [19] and by hypoxia [13]. Measurements may, perhaps preferably, be made on exhaled air at near-normal tidal volumes, thus sampling the later phase of the exhalate where carbon dioxide and oxygen have reached relatively stable concentrations. Since NO concentrations change with expiratory flow, the exhalation rate should either be controlled by a standardized restrictor or should be determined by flow measurement. It has been suggested that in adults, exhaled NO is determined at a flow rate of 100–300 $mL \cdot s^{-1}$ since this is relatively well tolerated in most individuals and since NO excretion is relatively constant over this flow range, whereas NO excretion drops markedly at lower flow rates, at least in adults [58]. Near-maximal expiratory flow rates can be accompanied by marked increments in NO excretion [59], and should probably also be avoided. In adults, using a standard commercial flow restrictor yielding a flow of 250 $mL \cdot s^{-1}$ at an airway pressure of 5 cmH_2O, exhaled NO concentration in normal subjects is regularly below 7 parts per billion (ppb), and the excretion rate during the single breath plateau is 100 $nL \cdot min^{-1}$ or less [58, and unpublished data]. In all subjects where a higher concentration or excretion rate has been encountered, a history of increased airway reactivity or overt asthma has on careful interview always been found. Since body size is more important for NO excretion rates than for exhaled concentration [60], it is evident that tables displaying values corresponding to normal subjects will have to be determined for excretion rates, relating them to anthropometric measures most relevant to respiratory system size.

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